Research Report

THE ULTRASTRUCTURE OF THE OTOLITH ORGANS IN SQUIRREL MONKEYS AFTER EXPOSURE TO HIGH LEVELS OF GRAVITOINERTIAL FORCE*

Heinrich H. Spoendlin, Harold F. Schuknecht, and Ashton Graybiel

Bureau of Medicine and Surgery
Project MR005.13-6001
Subtask 1 Report No. 102

NASA Order No. R-93

Released by

Captain H. C. Hunley, MC USN
Commanding Officer

*This research was conducted under the sponsorship of the Office of Life Science Programs, National Aeronautics and Space Administration.

5 November 1964

U. S. NAVAL SCHOOL OF AVIATION MEDICINE
U. S. NAVAL AVIATION MEDICAL CENTER
PENSACOLA, FLORIDA

SUMMARY PAGE

THE PROBLEM

The present experiment was designed to learn if animals, after exposure to levels of acceleration which might be encountered in launch and re-entry of spacecraft,reveal any changes in the ultrastructure of the otolith organs. Squirrel monkeys were chosen partly because they may be used in spaceflight and partly because the results may, with caution, be extrapolated to man. An important by-product of this investigation was the extension of our knowledge of the fine structure of the otolith apparatus in squirrel monkeys.

FINDINGS

Eight of eleven squirrel monkeys survived exposure to gravitoinertial force of either 5.43 or 10.92 G units for periods up to ten minutes in different body (head) positions. The nature of the head support was believed to be responsible in two of the deaths and headward (negative) acceleration in the third. Gross examination of the brains revealed no pathological changes. Following centrifugation, some of the monkeys manifested ataxia and other disturbances which disappeared in minutes or hours.

The ultrastructure of the maculae, as revealed by electronmicroscopy, was not altered in any of the animals exposed to high G stress. A detailed account of the findings in these and normal control animals is given and includes some new observations.

It was concluded that exposure to gravitoinertial forces greater than 10.92 G units is necessary before physical alterations in fine structure of the macula can be demonstrated in squirrel monkeys. The possibility was not ruled out that the clinical manifestations had their genesis in the semicircular canals. If the G loadings in this experiment are not exceeded in orbital space flights, alterations of the macula would be ascribable to other causes including the prolonged deafferentation associated with weightlessness.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of Edward L. Ricks in exposing the animals to centrifugation.

INTRODUCTION

The suppression of the otolith organs which has been shown to occur in weightlessness (1) raises the possibility that prolonged exposure to null gravity in space flight might lead to functional or even structural alteration in these organs. The experiments designed to study this problem in actual flight are complicated by the fact that the high accelerations on lift-off and re-entry might produce injury. Indeed, centrifugation has been used to "throw off" the otoconia in animal experiments (2-4), and human subjects have experienced ataxia for hours after "high-G" centrifuge runs (5-7). Accordingly, the present experiment was designed to learn if animals, after exposure to levels of acceleration which might be encountered in launch and re-entry of spacecraft, revealed any changes in the ultrastructure of the otolith organs. Squirrel monkeys were chosen, partly because they may be used in space flight and partly because the results may, with caution, be extrapolated to man. An important by-product of this investigation was the extension of our knowledge of the fine structure of the otolith apparatus in squirrel monkeys.

MATERIAL AND METHODS

Eleven squirrel monkeys were exposed either to 5.43 or 10.92 G units for one to ten minutes in different positions in the human centrifuge (Table I). Two other animals served as normal controls. Eight survived the exposure; half of these were sacrificed within fifteen minutes of the exposure and the other half twenty-four hours after exposure. Prior to sacrifice the left labyrinth of each animal was opened under general anesthesia and the maculae fixed in situ with cold osmic acid for electronmicroscopic studies. The right ear was then fixed by intracardiac perfusion with Heidenhain-Susa solution and used for light microscopic study. The brain was examined grossly with the dissecting microscope for evidence of pathological change.

RESULTS

Exposures to centrifugal forces of 10.92 G units were tolerated for periods of one minute. After a one-minute exposure some animals were severely ataxic for several minutes or hours but always recovered completely. The lower level of acceleration (5.43 G units) was tolerated for as long as ten minutes, although the animals differed in their reactions. One monkey was given three minutes' centrifugation without ill effects but appeared paralyzed for several minutes after seven more minutes of exposure and was then ataxic for several hours.

Macroscopic examination of the brains of animals which died from lethal centrifugation revealed no pathologic changes. The cause of death was ascribed to the character of the head support used in the first two experiments (CB and CC) and then discarded; headward acceleration was a likely cause of death in the case of monkey CG.

Table I

Experimental Method Used in Exposing Squirrel Monkeys to Gravitoinertial Force in Human Centrifuge

Monkey	Magnitude Resultant Force (G units)	Duration Exposure (Minutes)	Force Vector with Respect to Skull Exit	Survival Time
CB	10.92	10	L ear	-*
CC	10.92	5	R ear	-*
CA	10.92	1	L ear	15 min.
CE	10.92	1	L ear	24 hrs.
CF	10.92	1	R ear	15 min.
CD	10.92	1	R ear	24 hrs.
CI	10.92	1	Base	15 min.
CH	10.92	1	Base	24 hrs.
BB	5.43	10#	L ear	15 min.
CG	5.43	3	Vertex	-*
CJ	5.43	10#	R ear	24 hrs.

*Animals died under exposure; CB and CC probably due to character of head support, CG to "negative G."

#Exposure in two stages: 3 min., pause, 7 min.

Light microscopic studies failed to reveal structural changes (Figure 1); so, we directed our attention to a study of the ultrastructure of the vestibular sensory epithelium and possible changes which might result from G stress. Again no differences could be found between the maculae of animals subjected to high intensities of centrifugal force and those of normal control animals. A description of the morphology of these structures in the squirrel monkey has not been presented before and is considered to be of sufficient importance to record.

MORPHOLOGY

The sensory cells of the maculae of the squirrel monkey have the same basic structure as other mammals (8-10). Two types of hair cells are evenly distributed throughout the macula (Figures 2 and 3) unlike the crista where, according to Wersäll (8), the hair cells of type I are mainly at the summit and the hair cells of type II at the slopes of the crista.

The bottle-shaped cell (type I) is surrounded by a nerve chalice except at the apex (Figure 2). The narrow bottle neck can be very long. It contains a large number of longitudinal tubules having the same appearance as axonal neurotubules suggesting a similar function, that of conduction of the nerve impulse (Figure 4). The similarity of ultrastructural elements in type I hair cells and in neurones suggests that sensory cells belong to the same family as neurones (11). Other cytoplasmic structures, such as mitochondria and endoplasmic membranes, are very rare in the neck region of the cell. They are concentrated either in the apical or in the basal part of the cell.

Type II hair cell has a more irregular cylindric shape (Figure 3). It usually extends through the entire thickness of the sensory epithelium with the basal nucleus at the bottom. The density of the cytoplasm varies considerably. In some cells it is very dense and is rich in ribosomes, Golgi membranes, and vescicles. Others have a lighter cytoplasm, similar to that of type I hair cells. Instead of one nerve chalice, such as exists for type I hair cells, the type II has several smaller independent nerve endings in contact with the basal part of the cell.

The apex and the bodies of the sensory cells, the synapses, and the nerve fibers are the most probable sites for functional steps in the transformation of the mechanical response into the nerve impulses. These areas contain structures which could be expected to change under G loading conditions of stress.

The appearance of the sensory hairs in animals exposed to G stress is not different from that of normal animals (12). Each sensory cell carries from 60 to 100 stereocilia and 1 kinocilium, geometrically arranged. The kinocilium originates in a given group of sensory cells always at the same side of the cell surface at the periphery of the bundles of stereocilia (13). Occasional bending of the rather rigid stereocilia is also found in normal control animals.

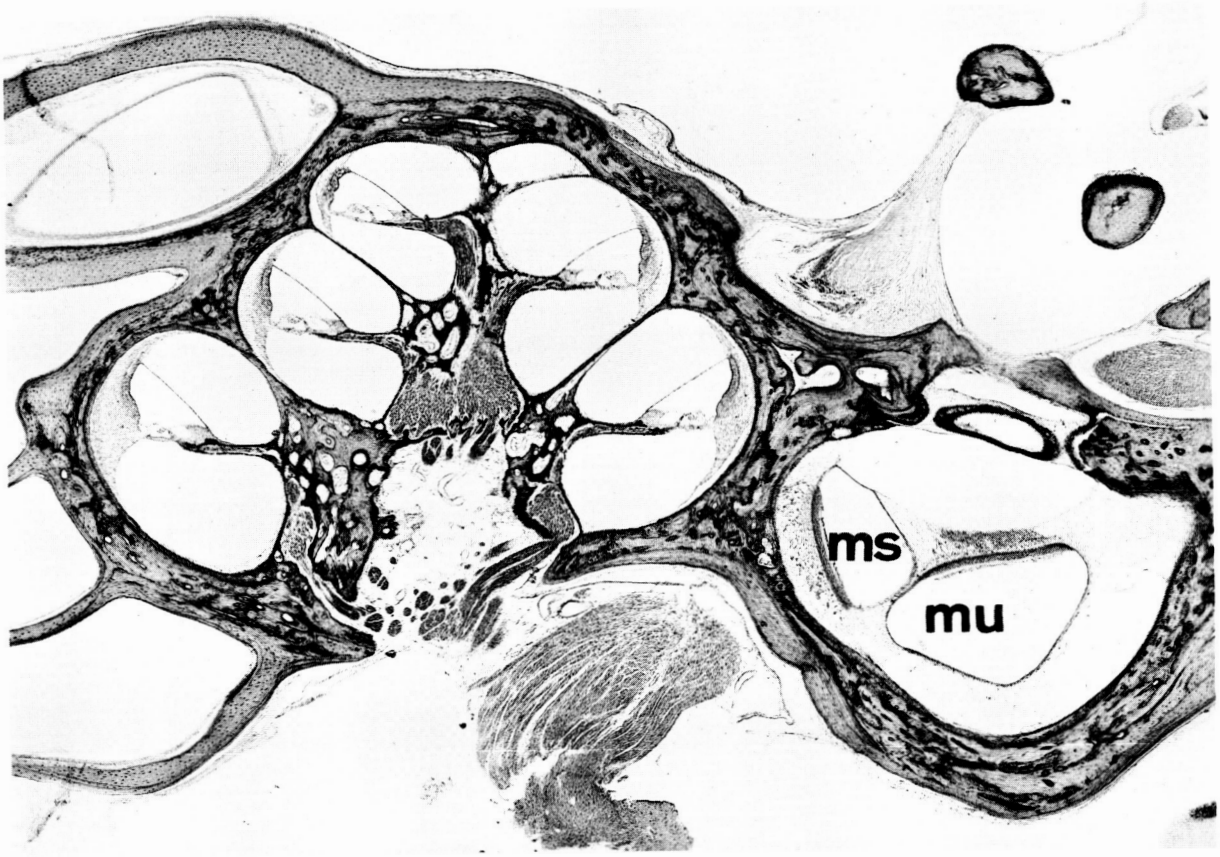
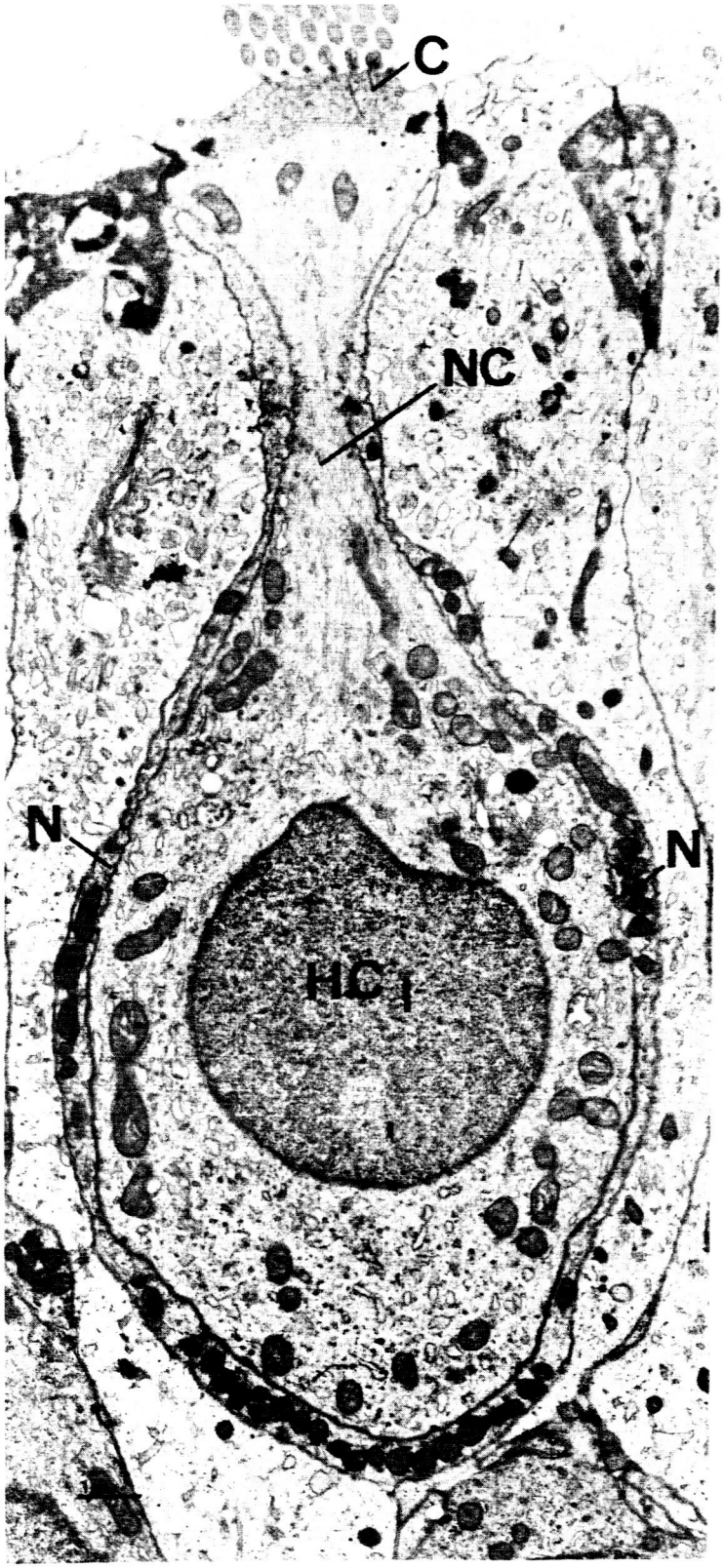


Figure 1

Horizontal section through a temporal bone of a monkey which was sacrificed immediately after an exposure to 10.92 G units for one minute.

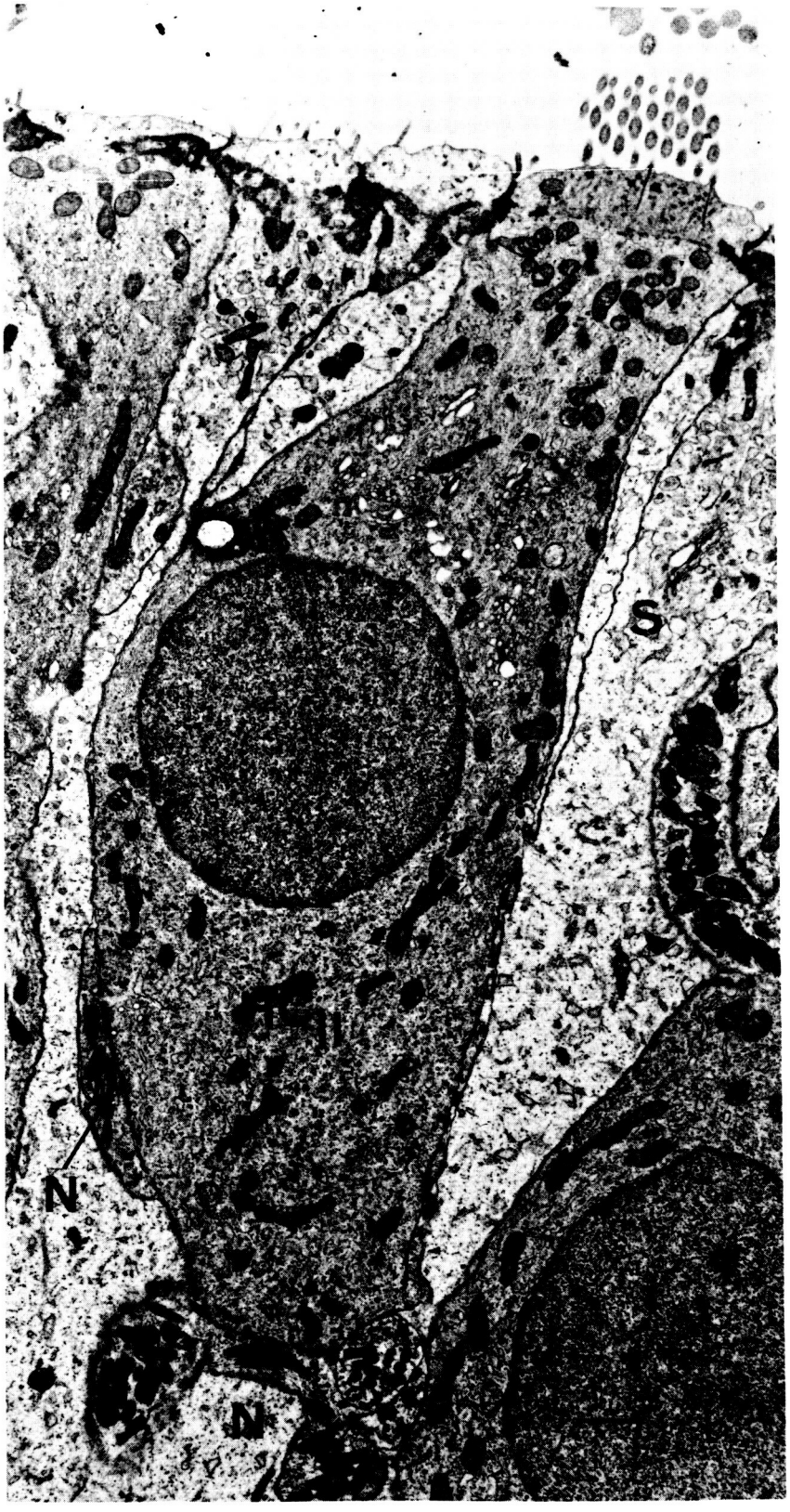
There are no anatomical alterations in the inner ear. Macula utriculi (MU) and sacculi (MS) are intact with the otoliths in place.



Electronmicroscopic picture of a hair cell type I (HC I) in a macula utriculi from an exposed monkey (exposure 10.92 G for one minute; survival time 24 hours). Normal ultrastructural features of this bottle-shaped cell are surrounded by a nerve chalice (N). The narrow neck (NC) of the hair cell with longitudinal fibrils of tubules is in a transverse section in Figure 4.

C = cuticula with stereocilia.

Figure 2



A hair cell of type II (HC II) of a macula utriculi from an exposed monkey (exposure 10.92 G for one minute, survival time 24 hours) with a very dense cytoplasm (compare hair cell Type I, Figure 2) embedded in supporting cells (S) with some individual nerve endings (N) at the base.

Figure 3

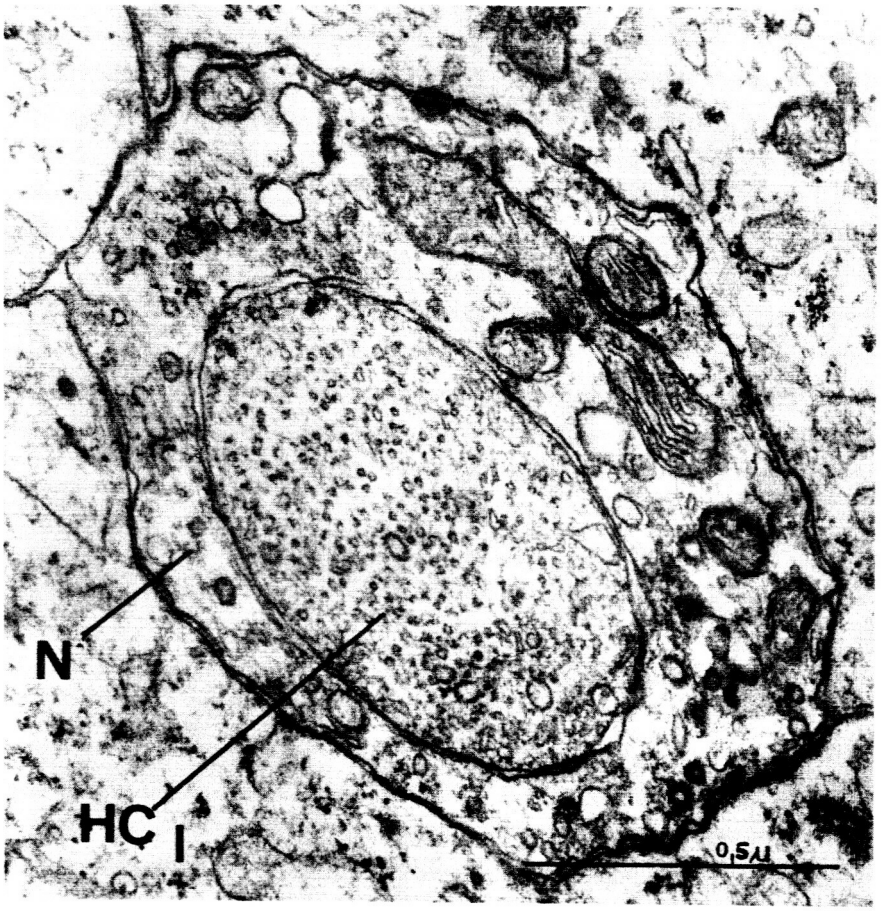


Figure 4

Horizontal section through the neck of hair cell type I (HC I) of a macula utriculi from a normal monkey. The hair cell is surrounded by the nerve chalice (N). There is a great number of tubules (resembling neuro-tubules) within this part of the sensory cell, but no mitochondria or other cytoplasmic organelles.

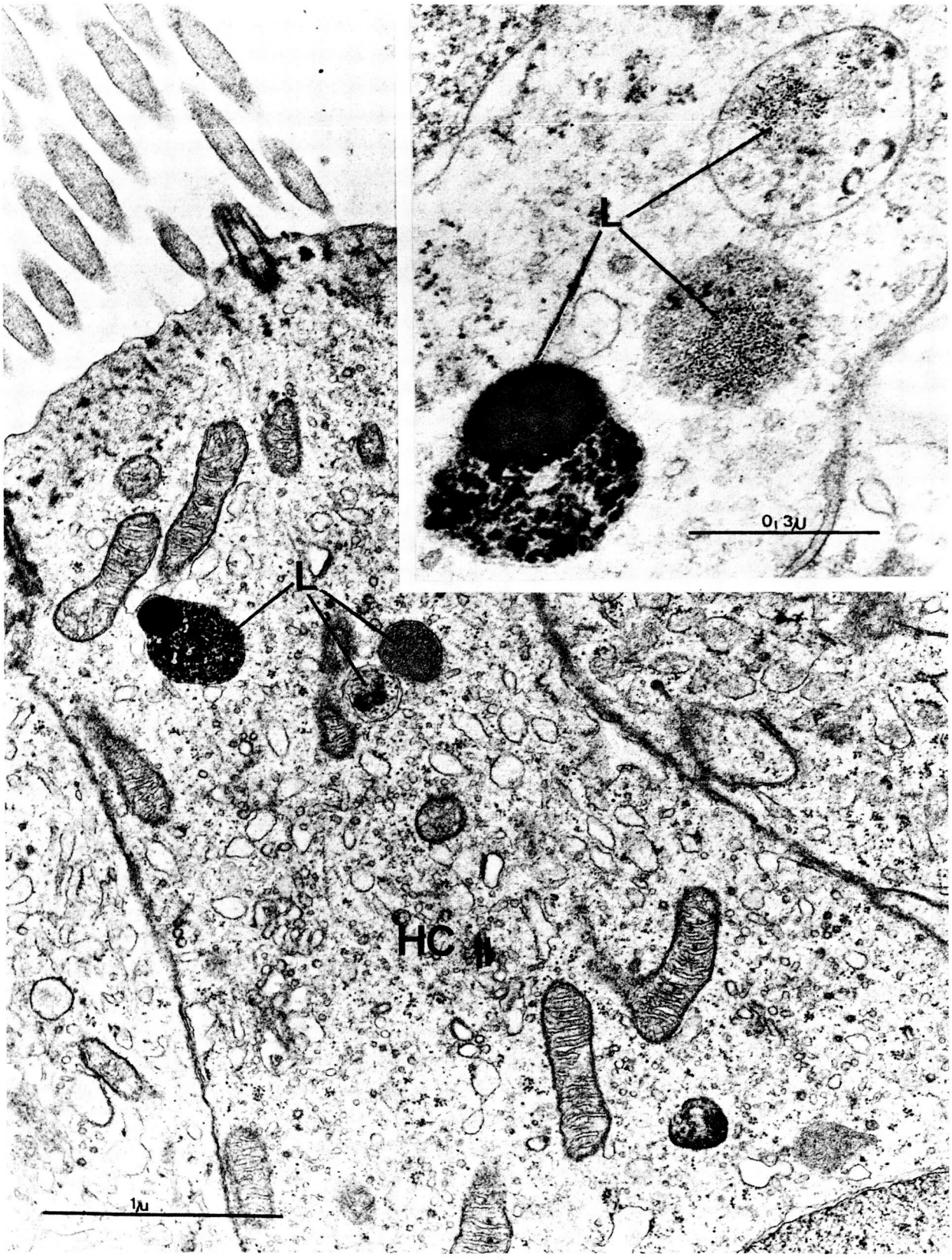


Figure 5

Figure 5

Legend

Apical part of a hair cell type II (HC II) in a macula of an exposed monkey (exposure 5.43 G for ten minutes, immediately sacrificed). Different types of lysosomes (L) as they are frequently seen in sensory and supporting cells of normal and exposed animals.

Inset: A group of lysosomes from a macular sensory cell of a normal monkey. The fine granular inclusions seem to be condensed to large osmiophilic masses in the lower lysosomes. The upper lysosome contains a few vesicles as if it were derived from a multivesicular body.

Apical protoplasmic protrusions, described by Engström and Wersäll (9) in guinea pigs and cats, extend between the sensory hairs of the sensory cells as small balloons of cytoplasm. In monkeys they are not so large and abundant as in guinea pigs. Their significance is not known. Engström et al. (14) have suggested the possibility that they might be a preparation artifact. Their very regular appearance in maculae prepared under different conditions suggests, however, that they are of physiologic importance. They show no changes after high G exposure.

The cytoplasm of the sensory cells in general is rich in Golgi membranes, small vesicles, vesicular bodies, and ribosomes. Numerous black inclusions in the sensory and supporting cells have the structural characteristics of lysosomes (Figure 5). In addition, we frequently see condensation of cytoplasm with a wispy appearance in the infranuclear part of the hair cells, a finding not previously described in the sensory cells of the inner ear (Figure 6).

The significance of lysosomes is not known. Histochemical studies indicate that they contain a number of hydrolases, anatomically separated from the surrounding cytoplasm (15). Morphologically they are vaguely defined as bodies, delimited by a single membrane (unit membrane) and frequently containing ferritin-like inclusions(16). In our specimens they appear in different forms but correspond to the morphological definition of lysosomes; that is, bodies with a single limiting membrane, usually filled with osmiophilic substances. There are lysosomes of homogeneous appearance, others are finely granulated or filled with osmiophilic clumps, and still others consist entirely of a black mass. All those forms of inclusions can appear in one lysosome, suggesting that there is an evolution in the formation of those inclusions. The extremely fine granules may gradually condense to clumps and finally to a coherent osmiophilic mass or vice versa (Figure 5). Black granular inclusions of the same type also can be observed in structures similar to multivesicular bodies. Again, there is no apparent difference between the lysosomes of normal and exposed animals.

It is generally believed that impulse transmission from the sensory cell to the nerve ending occurs only in certain areas with specific synaptic structures. The morphological variants of such synaptic structures within the maculae are striking. The most regularly observed probable synaptic structure is located where axon and cell membrane are parallel, with a narrowing of the intercellular space to a constant width of $\sim$ 150 Å. The cytoplasm of the axon and of the sensory cell in syanptic regions frequently shows a condensation which seems to extend into the synaptic cleft (Figure 7 A), similar to the demosomes of epithelial cells. Adjacent to such probable synaptic zones the intercellular space frequently is considerably widened (Figure 7 B).

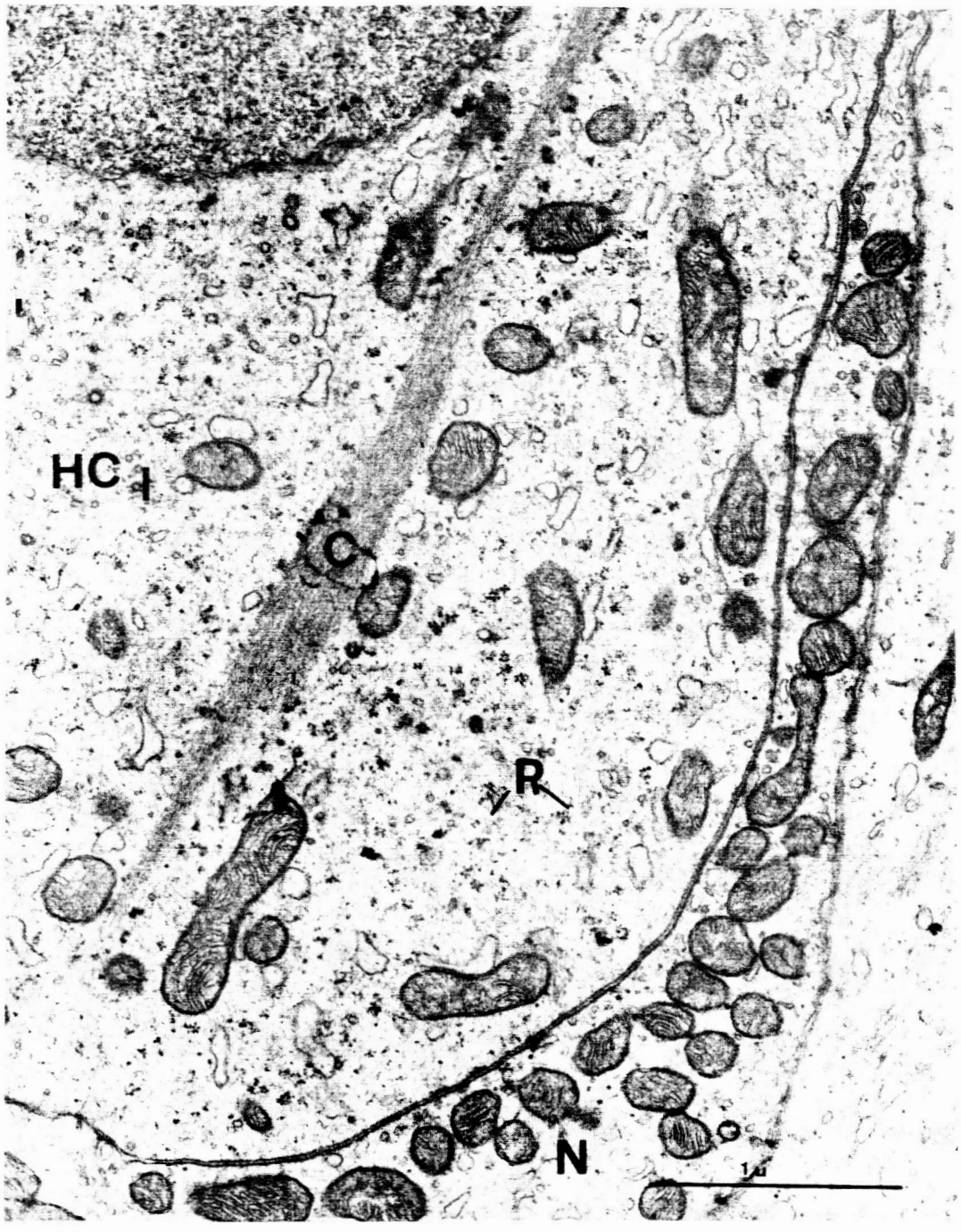


Figure 6

Basal part of a hair cell type I (HC I) in a macula of an exposed monkey. Wispy cytoplasm condensation (C) and a large number of ribosomes (R) in the cytoplasm of the sensory cell. There are no ribosomes in the nerve chalice (N).

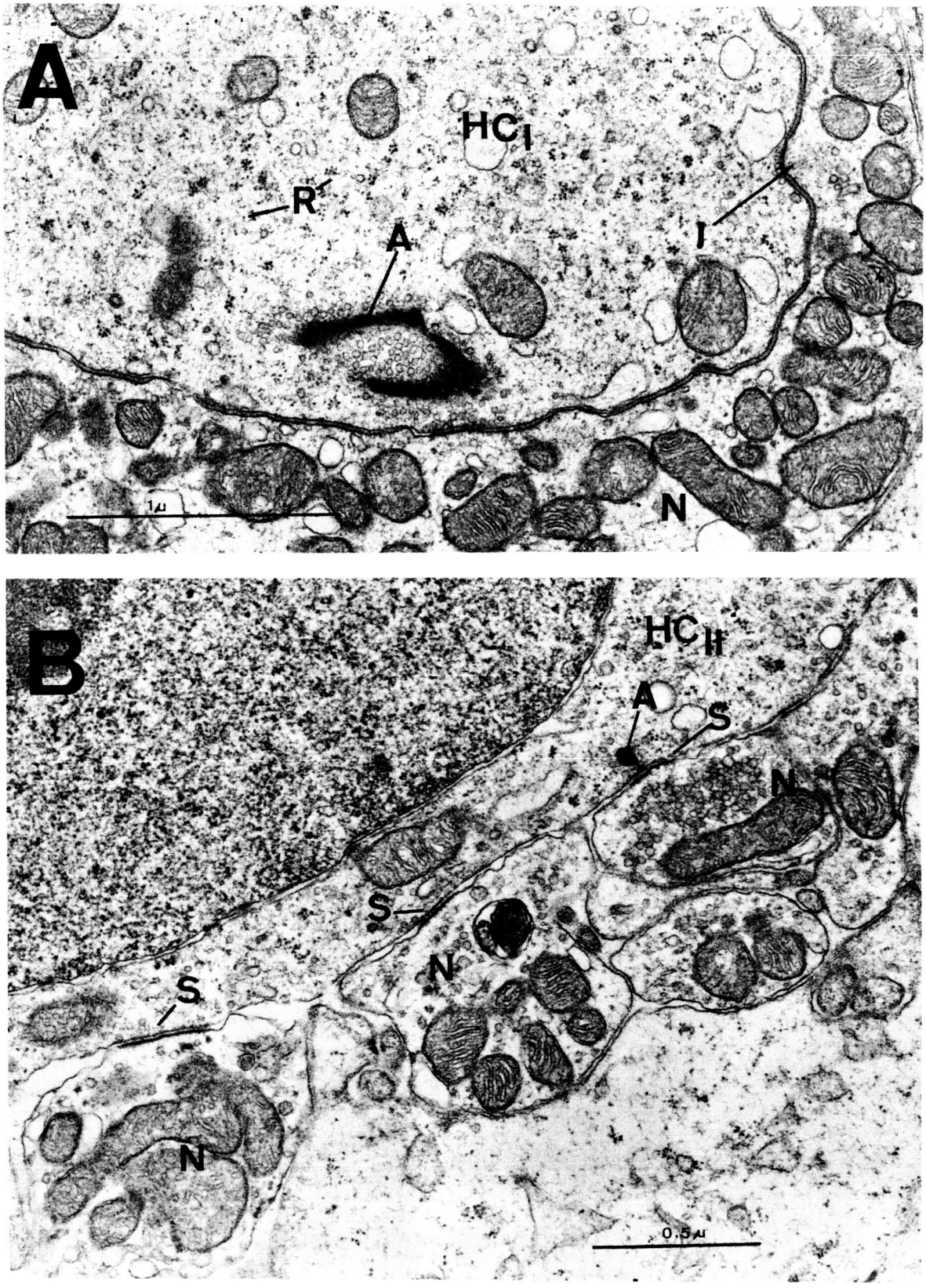


Figure 7

Figure 7
Legend

A. Basal part of a hair cell type I (HC I) with a big accessory synaptic structure with many synaptic vesicles (A). At the site of a beginning invagination (I) of the nerve chalice (N) is a narrow synaptic cleft, where sensory cell-plasma membrane and axon-membrane of the nerve chalice are very close. Many ribosomes (R) in the sensory cell, but none in the nerve chalice (N).

B. A group of nerve endings (N) at the base of a hair cell type II (HC II) with synaptic area (S) and accessory synaptic structure (A) in the sensory cell. The correspondent nerve ending contains many synaptic vesicles. Between the synaptic areas the gap between sensory cell and nerve ending is frequently widened.

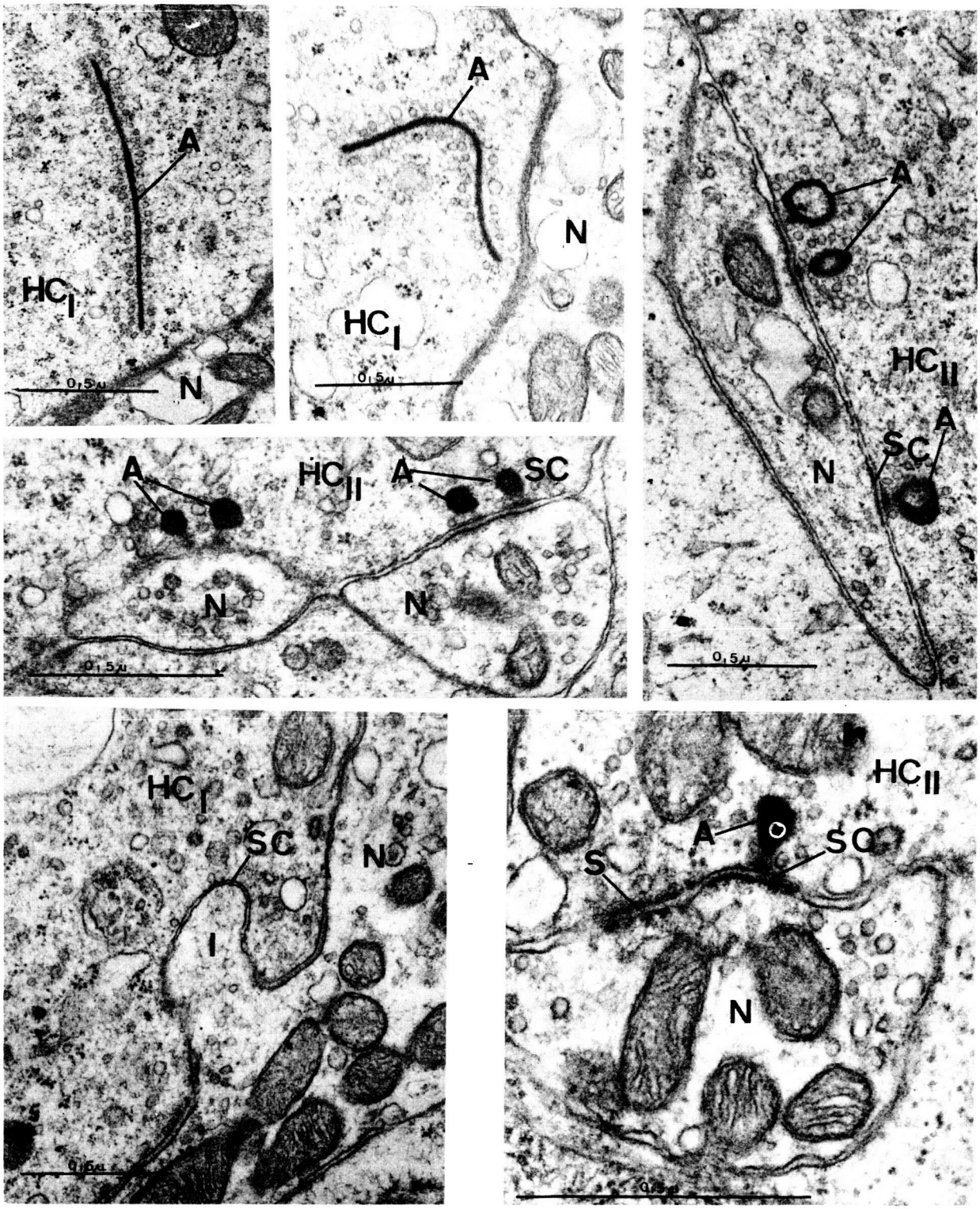

A
HC I
N
0,5 µ
A
N
HC I
0,5 µ
A
HC II
SC
N
0,5 µ
A
HC II
A
SC
N
N
0,5 µ
HC I
SC
N
I
HC II
A
SC
S
N

Figure 8

Figure 8
Legend

Different types of accessory synaptic structures (A) in hair cells type I (HC I) and type II (HC II) as observed in normal and exposed animals. The synaptic cleft (SC) shows a constant width of about $\sim$ 150 Å. Deep invaginations (I) of the nerve chalice (N) into the hair cell type I might act as a synapse too.

In addition to this basic structure, there is a variety of accessory synaptic structures in the cytoplasm of the sensory cells. All of them have one common feature: They show a very dense osmiophilic structure, frequently srrrounded by small vesicles of the dimension of synaptic vesicles (17). The most commonly found formations are synaptic bars similar to those in the organ of Corti as described by Smith and Sjöstrand (18). There are also spherical structures which appear as rings, or large, long, straight, or bent laminas, extending far into the cytoplasm of the sensory cell. These spherical structures can be found only in the hair cells of type I, whereas all other structures appear in both types of sensory cells. An interesting finding is invaginations of the nerve chalices into the bodies of the sensory cells of type I, where the intercellular spaces are narrow but where no accessory synaptic structures can be seen (Figures 7 and 8). The number of synapses per sensory cell and nerve ending is found to vary greatly in both normal and G-stressed animals. In serial sections we find some cells with many and others with no detectable synaptic structures.

In the nerve chalices of the hair cells of type I we find no synaptic vesicles, neurofilaments, or neurotubules. Some of the bud-like endings at the hair cells of type II are filled with vesicles similar to the cochlear efferent endings. However, the morphologic distinction between efferent and afferent fibers is not so clear as in the cochlea (19,20). Nerve endings with synaptic vesicles are frequently associated with either synaptic bars or an accumulation of synaptic vesicles on the sensory cell side of the synapse (Figure 7). It has been pointed out (21,22) that they might be substrates for transmitter substances, such as acetylcholine. It seems doubtful, however, that transmitter substances would be present on both sides of the synapses. Synapses between a vesiculated nerve ending and another nerve fiber or nerve chalice are rare.

The nerve fibers vary greatly in diameter. Large fibers of 5 u diameter usually end as a nerve chalice around the hair cells of type II. After leaving the myelin sheath under the basement membrane, the bare axons penetrate the sensory epithelium. They are intimately surrounded and embedded in deep grooves in the supporting cells (Figure 9 A).

The axons are rich in mitochondria. Both neurotubules and neurofibrils can be identified in the same axon. Usually they do not extend into the nerve endings or nerve chalices. The lack of ribosomes in the axon and nerve endings is remarkable, whereas they are abundantly present in the supporting and sensory cells (Figures 6 and 7).

The mitochondria in the large nerve fibers and endings of both normal and exposed animals appear in two forms: The more numerous type is elongated, sometimes having a ramified shape and a homogenous dense stroma interrupted by a large number of cristae; the other type is large and rounded with a light stroma and typical cristae (Figure 9 B). It is unlikely that the latter are the result of fixation since they are irregularly distributed among a large number of usual mitochondria, and they appear only within the nerve fibers and nerve chalices.

DISCUSSION

Gravitoinertial centrifugal forces of 5.43 and 10.92 G units for periods of one to ten minutes produced no morphological changes in the end organs of the gravireceptors in the macula.

The absence of any structural change in the otolith organs under the conditions of this experiment raises two questions : 1) At what level of force will the first indications of injury appear, and 2) what caused the ataxia in some of the monkeys?

With regard to the first, Wittmaack (2), de Kleyn and Versteegh (3), and Hasegawa (4) damaged the maculae by experimentally centrifuging animals at high speeds for short durations of time. The damage, which consisted of throwing the otoliths off the maculae, occurred only after exposure to forces greater than 100 G units (1 G unit = normal gravitational force). On the other hand, they found no changes in the inner ears of animals exposed to moderate gravitational force for long periods of time. Such evidence of gross injury, however, does little to define the level where the first indications of pathological distrubance occur.

The post-stimulation ataxia cannot be attributed to end organ changes in the otolith organs but might have had its genesis in the semicircular canals.

The close parallel in the manifestation of ataxia between centrifuged human and animal subjects is of more than passing interest. Disappearance of the ataxia suggests either that the injury was quickly reversible or that any residuum was compensated by the redundancy of nature. Some of the cellular structures, such as lysosomes, synaptic structures, and mitochondria, appeared to exist in different forms, a condition which might be related to the functional state of the cells. From our studies we could not determine whether states of altered function were reflected in the appearance of these structures.

Whereas a previous study showed increasing numbers of osmophilic inclusions in the hair cells of the cochlea with auditory stimulation (23,24), there is no difference in the osmophilic granules in the hair cells of the maculae of G-stressed and unstressed monkeys.

The relation of lysosomes to Golgi membranes and multivesicular bodies has been discussed by many authors (16,25,26). de Duve (15) considered lysosomes to be organelles of intercellular digestion,containing proteolytic enzymes which, under normal conditions, are anatomically separated from the cytoplasm of the cell, thereby preventing auto-digestion. Many consider the dark inclusions as accumulations of metabolic endproducts (slags) which cannot be eliminated by the cell. Most authors believe that lysosomes play an important role in the metabolism of the cell, although their exact significance is not yet known.

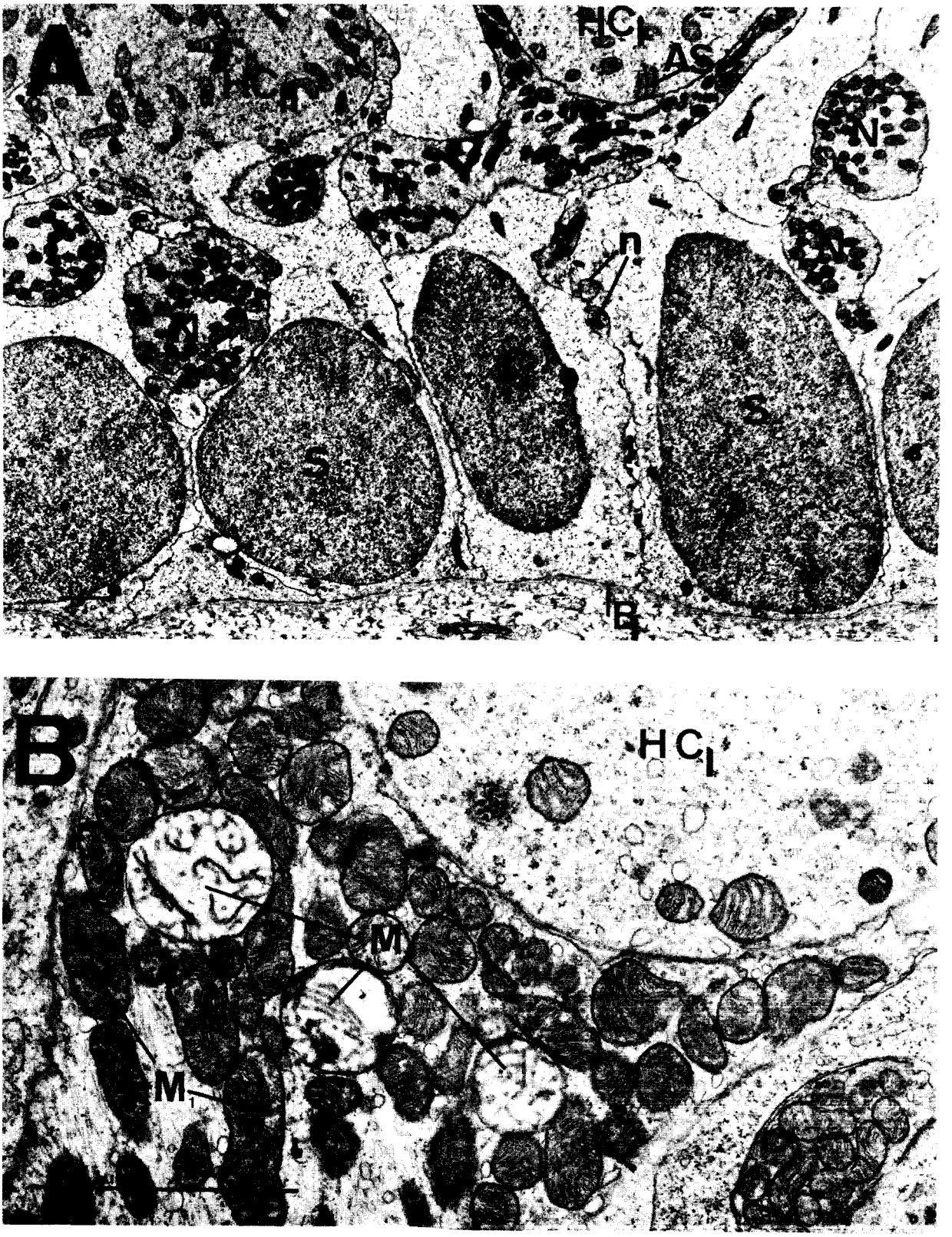


Figure 9

Figure 9

Legend

A. Basal part of a macula of an exposed monkey. Between the supporting cells (S) which sit on the basal membrane (B) are many large (N) and some small nerve fibers (n). Within the base of a hair cell type I (HC I) is a pronounced triple accessory synaptic structure (AS).

Hair cell of type II = HC II

B. Two types of mitochondria in a nerve chalice as found in normal and exposed animals. Among normal appearing mitochondria (M_1) are some swollen mitochondria (M_2) with a very light stroma.

Because of the lethal effect, it is not possible to stimulate the gravity receptors to the same magnitude as is possible for acoustic receptors.

The synapses between sensory cells and nerve endings must contribute an important link in the functional chain within the receptor organ. Although it has been postulated that electrical impulse-transmission can occur at synapses, the transmission is more commonly considered to be chemical (11). In the CNS, different types of axodendritic and axosomatic synaptic areas have been identified, such as the synaptic membrane complex (SMC) and a desmosome-like structure with or without adjacent synaptic vesicles (27,28). deLorenzo (29) found in a combined electronmicroscopic-histochemical study a much higher acetylcholinesterase activity within the axodendritic synapses with cytoplasmic condensation than within the axosomatic synapses which show much less structural differentiation. Such findings support the concept that at least some of the specific structures at neural junctions really are sites of synaptic transmission. This is not proven, however, and the fact that very similar structures (desmosomes) are found between supporting or other cells (30) may mean that the desmosome-like "synaptic structures" have the same function as desmosomes, that is, to hold the areas of neural junction together (22).

Accessory synaptic structures such as synaptic bars, accessory membranes, or synaptic vesicles are restricted to neural junctions. The large variety of synaptic structures in the maculae of the squirrel monkey is striking. The variation from the basic desmosome-like structure up to large accessory structures has not been described in the vestibular sensory epithelia of other animals. It might be related to the functional importance of the gravity receptors in squirrel monkeys where acrobatic skill is important to survival. The special importance of the vestibular system in monkeys as compared to other animals is also expressed by the relative numbers of nerve fibers in the vestibular nerve. According to Gacek and Rasmussen (31), there are twice as many vestibular nerve fibers in the monkey as in the guinea pig.

It would be of interest to know if the morphologically different types of synapses are correlated with qualitatively different functions or whether they correspond to different functional states of the synapse. The variation in number of synapses per sensory cell suggests that synapses are not permanent structures but that they are built up and wear out in a functional cycle. Also, the difference in density of ribosomes, Golgi membranes, and vesicles within the cytoplasm of the sensory cell seems to indicate that not all sensory cells are in the same functional state at a given time. The different appearances of the mitochondria might also be the result of different states of mitochondrial function. In this regard, Packer (32) described a partly reversible mechanism of swelling and shrinking of the mitochondria in correlation with oxydative phosphorylation. The reason swollen mitochondria appear only within the nerve chalices and large nerve fibers may be related to the lack of ribosomes in those structures.

One thing seems certain: The variations in structural appearance of the sensory cells, cytoplasm, synapses, and mitochondria are not caused by G-stress for they also are present in normal animals.

REFERENCES

1. Miller, E. F., II, Kellogg, R. S., and Graybiel, A., Otolith organ activity within earth standard, one-half standard, and zero gravity environments. Presented at the 35th Annual Meeting, Aerospace Medical Association, Miami Beach, Florida, 1964.

2. Wittmaack, J., Ueber die Veränderungen im innern Ohr nach Rotationen. Verh. dtsch. otol. Ges., 19:150-156, 1909.

3. deKleyn, A., and Versteegh, C., Labyrinthreflexe nach Abschleuderung der Otolithenmembrane bei Meerschweinchen. Pflüg. Arch. ges. Physiol., 232: 454-465, 1933.

4. Hasegawa, T., Der Bau und die Funktion der Otolithenmembrane. Arch. Ohr-Nas-Kehlkopfheilk., 182:555-559, 1963.

5. Brown, J. L., and Lechner, M. G., Acceleration and human performance: A survey of our research. NADC-MA-5503. Johnsville, Penna.: U. S. Naval Air Development Center, 1955.

6. Duane, T. D., Beckman, E. L., Ziegler, J. E., and Hunter, H. N., Some observations on human tolerance to exposure of 15 transverse G. NADC-MA-5305. Johnsville, Penna.: U. S. Naval Air Development Center, 1953.

7. Frenkel, O. M., Ueber die Aenderungen der Erregbarkeit des Vestibularapparates unter dem Einfluss wiederholter Reizung. Bull. Biol. Med. U.R.S.S., 9: 69-72, 1940.

8. Wersäll, J., Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig; a light and electron microscopic investigation. Acta otolaryng., Stockh., Suppl. 126, 1-85, 1956.

9. Engström, H., and Wersäll, J., The ultrastructural organization of the organ of Corti and of the vestibular sensory epithelia. Exp. Cell. Res., Suppl. 5, 460-492, 1958.

10. Bairati, A., Jr., Récentes études des structures submicroscopiques des organs du vestibule. Acta otolaryng., Stockh., Suppl. 163, 9-25, 1961.

11. Davis, H., Some principles of sensory receptor action. Physiol. Rev., 41: 391-416, 1961.

12. Spoendlin, H., Organization of the sensory hairs in the gravity receptors of the squirrel monkey. Z. Zellforsch., 62: 701 , 1964.

13. Spoendlin, H., Ueber die Polarisation der vestibulären Sinneshaare. Practica, in press, 1964.

14. Engström, H., Ades, H. W., and Hawkins, J., Structure and functions of the sensory hairs of the inner ear. J. acoust. Soc., 34: 1356-1363, 1962.

15. de Duve, C., Lysosomes organelles of intracellular digestion. Exp. Cell. Res., Suppl. 7, 169, 1959.

16. Novikoff, A. B., Lysosomes and related particles. In Brachet, J., and Mirsky, A. E. (Eds.), The Cell. Volume II. New York: Academic Press, 1961.

17. de Robertis, E., Submicroscopic morphology of the synapse. Int. Rev. Cytol., 8: 61-96, 1959.

18. Smith, C. A., and Sjöstrand, F. S., A synaptic structure in the hair cell of the guinea pig cochlea. J. Ultrastruct. Res., 5:184-192, 1961.

19. Kiumura, R., and Wersäll, J., Termination of the olivo-cochlear bundle in relation to the outer hair cells of the organ of Corti in guinea pig. Acta otolaryng., Stockh., 55:11-32, 1962.

20. Spoendlin, H., and Gacek, R., Electronmicroscopic study of the efferent and afferent innervation of the organ of Corti in the cat. Ann. Otol., etc., St. Louis, 72:660-686, 1963.

21. de Robertis, E., and Bennet, H. S., Some features of the submicroscopic morphology of synapses in frog and earth worm. J. biophys. biochem. Cytol., 1:47-58, 1955.

22. Robertson, J. D., The ultrastructure of a reptilian myoneural junction. J. biophys. biochem. Cytol., 2:381-394, 1956.

23. Spoendlin, H., Submikroskopische Veränderungen am Corti'schen Organ des Meerschweinchens nach akustischer Belastung; vorläufige Mitteilung. Pract. otorhinolaryng., 20:197-214, 1958.

24. Spoendlin, H., Ultrastructural features of the organ of Corti in normal and acoustically stimulated animals. Ann. Otol., etc., St. Louis, 71:657-677, 1962.

25. Benedetti, L. E., and Leplus, R., Cytologie de l'erythroblastose aviare; étude au microscope électronique. Rev. hémat., 13:199-230, 1958.

26. Farquhar, M. G., Wissig, S. L., and Palade, G. E., Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. J. exp. Med., 113:47-66, 1961.

27. Palay, S. L., The morphology of synapses in the central nervous system. Exp. Cell Res., Suppl. 5, 275-293, 1958.

28. Robertson, J. D., Bodenheimer, T. S., and Stage, D. E., The ultrastructure of mauthner cell synapses and nodes in goldfish brains. J. Cell Biol., 19:159-199, 1963.

29. de Lorenzo, A. J. D., Electron-microscopy of the cerebral cortex. I. The ultrastructure and histochemistry of synaptic junctions. Bull. Johns. Hopk. Hosp., 108:258-279, 1961.

30. Brightman, W. M., and Palay, S. L., The fine structure of ependyma in the brain of the rat. J. Cell Biol., 19:415-439, 1963.

31. Gacek, R. R., and Rasmussen, G. L., Fiber analysis of the statoacoustic nerve of guinea pig, cat, and monkey. Anat. Rec., 139:455-463, 1961.

32. Packer, L., Size and shape transformations correlated with oxidative phosphorylation in mitochondria. I. Swelling-shrinkage mechanisms in intact mitochondria. J. Cell Biol., 18:487-494, 1963.